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ABSTRACT

The hypothalamo-pituitary-adrenal (HPA) axis plays a key role in the neuroendocrine response to stress and in maintaining physiological homeostasis. However, stress that is chronic in nature can lead to HPA axis dysfunction and increase the risk for developing affective disorders, particularly if the stress is experienced during vulnerable periods in life. Sex differences in how the HPA axis responds to stress are well established, with females typically displaying heightened responses. The underlying cause of these sex differences are important to understand, as many neuropsychiatric disorders disproportionately affect females.

Much research has provided evidence for gonadal sex steroids in underpinning sex differences in HPA axis responsivity, however we suggest that neuroactive metabolites of these steroids also play a key role in the brain in mediating sex differences in HPA axis responses to stress.

The relationship between neuroactive steroids and stress is complex. Acute stress rapidly increases neuroactive steroid production, which can in turn modulate activity of the HPA axis. However under chronic stress conditions, stress can impact the brain's capacity to generate steroids, and this in turn has corollary effects on HPA axis function that may increase the propensity for psychopathology, given both HPA axis dysfunction and deficits in neuroactive steroids are implicated in affective disorders. Hence here we review the evidence from animal and human studies for sex differences in the interactions between neuroactive steroids and the stress axis at various stages of life, under physiological and pathophysiological stress conditions and consider the implications for health and disease.

INTRODUCTION

Although the term "stress" seemingly carries negative connotations, it represents an important physiological adaptation that is essential for an organism's survival. The concept of "stress" was proposed by Walter Cannon (Cannon, 1914) and Hans Selye (Selye, 1936) as a process that brings about physiological adaptations that allow an organism to cope with changes in the environment or its internal milieu. More recently, stress has been viewed as part of a framework known as "allostasis", defined as the process of "attaining stability through change" (McEwen, 2000). The typical response to stress involves activation of the sympathetic nervous system and the hypothalamo-pituitary-adrenal (HPA) axis, producing primary "allostatic mediators" such as catecholamines and glucocorticoids, which act on a range of systems to alter the organism's physiology and behaviour (Chrousos & Gold, 1992). As stress responses are dynamic, they additionally involve several other "allostatic mediators" including neurotransmitters, neuropeptides and steroids in the brain (i.e. neuroactive steroids), which interact with each other in a non-linear manner. An alteration in one mediator may therefore lead to knock-on effects in other mediators, and the end product is a stress response with variable characteristics, depending on the nature, severity, and duration of the stressor. Notably, stress that is chronic, severe or experienced during vulnerable periods in life is viewed as a physiological burden or "allostatic load", which may lead to the dysregulation of certain mediators, and in turn, an increased risk of an organism developing stress-related pathologies. The sex of an individual adds an additional layer of complexity to the dynamic nature of stress responses, as males and females exhibit marked differences in the extent of their stress responses, and critically in the incidence of stress-related disorders.

Sex differences in stress responses are attributed to the effects of sex-specific genes, as well as the effects of gonadal hormones (e.g. testosterone, estradiol, and progesterone) acting in the periphery or in the brain to exert their effects. The "organisational-activational hypothesis" of hormone-driven sexual differentiation (Phoenix *et al.*, 1959) posits that masculinisation of neural circuits associated with sexual behaviour results from differential exposure to testicular hormones both early in development and during adulthood. However, how exactly these gonadal steroids exert their action in the brain to mediate processes, especially those not directly associated with reproductive behaviour (e.g. stress responses), have not been fully

elucidated. This is further complicated by the complexity in the regulation of steroid action in the brain, as the brain is also a steroidogenic organ and has the capacity to produce or further metabolise steroids, especially in response to stress. These steroids that are produced endogenously in the brain are termed "neurosteroids", and have properties that are specific for their action in the brain, such as the ability to rapidly modulate neuronal transmission (Baulieu, 1991; Rupprecht, 2003). However, due to the difficulties in establishing the source and actions of steroids found in the brain, the umbrella term "neuroactive steroids" is more commonly used to refer to all steroids found in the brain that are able to regulate neural functions (Melcangi *et al.*, 2008). Neuroactive steroids (which by definition, includes gonadal steroids and adrenocorticosteroids that enter the brain) therefore lie at the intersection of sex differences and the modulation of stress responses.

This review will first describe sex differences in stress responses, with a particular focus on the HPA axis, due to the observation that HPA axis dysregulation underlies many stress-related psychopathologies (McEwen, 2003; Pariante & Lightman, 2008; Fernandez-Guasti *et al.*, 2012; Altemus *et al.*, 2014; Rincón-Cortés *et al.*, 2019). The role of gonadal steroids in establishing sex differences in HPA axis responsivity will be discussed, with an emphasis on the metabolism of these gonadal steroids to their downstream neuroactive metabolites in the brain. Sex differences in how the brain responds to stress may account for the sex bias in the propensity to develop stress-related disorders, hence sex differences in the levels of neuroactive steroids will be compared during the physiological response to acute stress, as well as under chronic stress conditions and in psychiatric disorders. The bi-directional relationship between steroids and stress will be explored by considering not only the role of neuroactive steroids in establishing and maintaining differences in stress responsivity across the lifespan, but also the impact of pathophysiological stress during critical periods of development on the brain's capacity to generate neurosteroids, and the knock-on effects for HPA axis regulation and mood.

REGULATION OF THE HPA AXIS

The core components of the HPA axis include the medial parvocellular region of the paraventricular nucleus (mpPVN) of the hypothalamus, the anterior pituitary gland and the cortical region of the adrenal glands. Following stressful stimuli, neurones of the mpPVN are

activated and release the peptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) at the median eminence into the hypophysial portal blood system that supplies the anterior pituitary gland. CRH and AVP stimulate the synthesis and secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary corticotrophs into the general circulation. ACTH in turn stimulates the adrenal cortex to secrete glucocorticoids into the bloodstream (cortisol in humans and corticosterone in rats and mice). ACTH and corticosterone secretion display a circadian rhythm with lowest levels at the onset of the inactive phase (i.e. the morning in rats, the evening in humans) and highest levels at the onset of the active phase (i.e. the evening in rats, the morning in humans) (Meaney *et al.*, 1992; Atkinson *et al.*, 2006). Glucocorticoids function to redistribute energy across the body, promote processes related to energy mobilisation in the brain and skeletal muscles, but inhibit processes related to growth, development, reproduction and immunity (Munck *et al.*, 1984; Sapolsky *et al.*, 2000). Glucocorticoids act by binding to glucocorticoid (GR) and mineralocorticoid receptors (MR), both of which are expressed in the brain, but show differential regional expression patterns and binding affinities (de Kloet *et al.*, 1998). Glucocorticoids primarily signal via MR under basal conditions, and act through GR mainly in instances where higher concentrations are present, such as during stress, or during the peak of the circadian rhythm (de Kloet *et al.*, 1998). Given the widespread actions of glucocorticoids, the magnitude of their production (i.e. activation of the HPA axis), as well as the termination of their production (i.e. inhibition of the HPA axis) is tightly controlled.

Activation of the HPA axis relies largely on complex neural circuits and is stressor-dependent (Godoy *et al.*, 2018). Physical stressors such as inflammation, haemorrhage, or pain signal through direct noradrenergic and adrenergic projections originating from the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) of the brainstem to the PVN (Ulrich-Lai & Herman, 2009); whereas psychological stressors which require appraisal of an event as 'stressful' activate more complex pathways, relying heavily on signal processing by limbic structures such as the hippocampus, prefrontal cortex, and amygdala (Godoy et al., 2018; de Kloet et al., 2019). Communication between these brain regions rely on neural circuitries involving GABA and glutamate neurotransmission (Ulrich-Lai & Herman, 2009). Signals from different limbic regions are integrated at regions such as the peri-PVN, the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (mPOA), which directly innervate the PVN,

eventually resulting in the production of CRH and/or AVP in the mpPVN neurones (Ulrich-Lai & Herman, 2009; Bains *et al.*, 2015). Stress also leads to the production and secretion of other allostatic steroid mediators from the adrenal glands, such as deoxycorticosterone (DOC) and progesterone (Torres *et al.*, 2001; Reddy, 2006; Hueston & Deak, 2014).

Activation of the HPA axis is followed by inhibitory mechanisms to shut the axis down once the stress no longer poses a threat (Herman, 2013). Inhibition of the HPA axis is controlled by both rapid neuronal signalling mechanisms, as well as other slower genomic mechanisms that involve gene expression changes (Ulrich-Lai & Herman, 2009). One of the key inhibitory mechanisms regulating HPA axis activity is the negative feedback inhibition mediated by glucocorticoid binding to GR, directly in CRH-producing neurones of the PVN, as well as in anterior pituitary corticotrophs (Keller-Wood & Dallman, 1984; Uht *et al.*, 1988) and indirect inhibition via glucocorticoid actions on GR and MR expressed in other limbic structures, such as the hippocampus (Herman *et al.*, 2005). GABA neurotransmission is an important aspect of HPA axis inhibition, as the PVN is directly innervated by GABA projections from various brain regions, including the peri-PVN and the BNST (Cullinan *et al.*, 1993; Herman *et al.*, 2016). Notably, neuroactive steroids such as the progesterone metabolite allopregnanolone and corticosterone metabolite, tetrahydrodeoxycorticosterone (THDOC), concentrations of which both increase considerably following acute stress, positively modulate $GABA_A$ receptors via an allosteric binding site to potentiate inhibitory GABAergic signalling (Purdy *et al.*, 1991; Barbaccia *et al.*, 1996; Hosie *et al.*, 2006; Girdler & Klatzkin, 2007). Given these actions, increased allopregnanolone and THDOC following stress have been proposed to constitute a negative feedback mechanism which aids termination of the HPA axis response (Wirth, 2011; Crowley & Girdler, 2014; Gunn *et al.*, 2015; Brunton, 2016). Indeed, *in vivo* experiments in rats confirm that pretreatment with either allopregnanolone or THDOC significantly attenuates stress-induced increases in plasma ACTH and corticosterone concentrations, possibly through the inhibition of CRH-producing neurones in the mpPVN (Owens *et al.*, 1992; Patchev *et al.*, 1996; Brunton *et al.*, 2009), further supporting their inhibitory role in the regulation of the HPA axis.

Sex differences in HPA axis function under physiological stress conditions

Sex differences in HPA axis stress responses in rodents are well established, and have been extensively reviewed elsewhere (Goel *et al.*, 2014; Handa & Weiser, 2014; Oyola & Handa, 2017; Heck & Handa, 2019). Although some inconsistencies are reported, possibly due to different experimental conditions, in general, stress induces a more robust activation of the HPA axis in females than in males.

Sex differences in responses to acute stress in rodents

Under stress-free conditions, females generally show greater baseline plasma corticosterone concentrations (Kitay, 1961; Handa *et al.*, 1994) and more pulses in the circadian rhythm of corticosterone secretion, compared with males (Atkinson & Waddell, 1997; Goel *et al.*, 2014). However this difference is not reflected in the brain (Droste *et al.*, 2009); possibly due to the higher levels of corticosteroid-binding globulin (CBG) present in the female circulation (Gala & Westphal, 1965). While basal circulating ACTH concentrations generally do not show sex differences (Viau *et al.*, 2005; Goel *et al.*, 2014), greater *Crh* expression in the mpPVN is typically reported in females (Viau *et al.*, 2005; Iwasaki-Sekino *et al.*, 2009; Goel *et al.*, 2014). Following an acute stressor, activation of the HPA axis is typically greater in female rodents than in males, reflected by the secretion of greater absolute amounts of corticosterone into the circulation, at a faster rate than in males, greater neuronal activation (c-Fos expression) of the PVN neurones (Babb *et al.*, 2013), and greater increases in *Crh* expression (Iwasaki-Sekino *et al.*, 2009)(Fig. 1). In the periphery, the pituitary gland is more sensitive to stimulation by CRH and the adrenal glands are more sensitive to ACTH in females (Fig. 1), with the latter evidently mediated by estradiol actions (Figueiredo *et al.*, 2007; Goel *et al.*, 2014). In terms of the inhibition of the HPA axis, it seems that there is diminished GR-mediated negative-feedback control of the HPA axis in females, resulting in a slower resolution of the stress response (Burgess & Handa, 1992).

Sex differences in responses to acute stress in humans

Sex differences in cortisol responses to psychological stress are also reported in humans (Goel et al., 2014), however the direction of difference is less consistent than those observed in rodents (Kirschbaum *et al.*, 1992; Seeman *et al.*, 2001; Kudielka & Kirschbaum, 2005; Uhart *et al.*, 2006; Rao & Androulakis, 2017). These inconsistencies are compounded by the fact that

stress perception and general HPA axis function show far more inter-individual variability in humans (Goel *et al.*, 2014; Rao & Androulakis, 2017; van der Voorn *et al.*, 2017), and patterns of cortisol production do not always appear to correlate with subjective stress responses (Gillies & McArthur, 2010).

In the CRH-dexamethasone suppression test, the cortisol response is higher in women than in men (Heuser *et al.*, 1994; Kunugi *et al.*, 2006), indicating lower sensitivity to glucocorticoid-mediated negative feedback inhibition. However, in stressed-based tasks, such as the Trier Social Stress Test (TSST), which involves public speaking and a mental arithmetic task, some studies report no differences in plasma cortisol and ACTH responses between the sexes, while others report concentrations to be generally higher in males (Kudielka & Kirschbaum, 2005). On the other hand, women tend to show greater responsivity to social rejection, thus the HPA axis responses to stress in humans are likely to be very much stressor-dependent (Stroud *et al.*, 2002).

Mechanisms underlying sex differences in the response to physiological stress

Sex differences in physiological responses to stress can be attributed to the actions of gonadal steroids and the genetic sex of the organism (Becker *et al.*, 2005). The central concept of the organisational-activational effects of gonadal steroids, first proposed by Phoenix *et al.* (Phoenix *et al.*, 1959) states that sex differences in the brain and behaviour are permanently sexually differentiated by testosterone during early critical periods of development (i.e. "organisational effects"), and these 'organised' characteristics are brought to expression during adulthood due to the continued presence of these gonadal hormones (i.e. "activation" effects) (McCarthy et al., 2012, Arnold, 2009) (Fig. 2). Although the initial study specifically examined sexual behaviour, this framework can also be applied to other phenotypes that show sexual differentiation (Blaustein & McCarthy, 2009).

In male rats, a testosterone surge occurs around gestational day 18 and again during the hours shortly after birth, where levels are significantly higher than those measured in female siblings (Weisz & Ward, 1980). Male neonatally gonadectomised rats have greater circulating corticosterone concentrations and Fos activation in the mpPVN, both under basal conditions and after 30 min of restraint exposure (Bingham & Viau, 2008). These effects can be normalised

with testosterone treatment on postnatal day (PND) 1-5, but not during adulthood, indicating organisational effects of testosterone during the early postnatal period (Bingham & Viau, 2008). More recent studies have also proposed that "organisational" effects also occur during puberty, when there are further maturational changes in the neural circuitry of the brain, due to the epigenetic effects of gonadal hormones (Schulz *et al.*, 2009; Morrison *et al.*, 2014).

During adulthood, steroids present in the brain, exert direct "activational" effects on cellular function, which are only observed when the steroids are present. Generally, testosterone dampens, while estrogen potentiates HPA axis function (Goel *et al.*, 2014; Handa & Weiser, 2014; Heck & Handa, 2019). Hence removal of the testes (and thus the main source of androgens) increases basal corticosterone levels, as well as stress-induced corticosterone secretion in males to "female-like" levels; whereas testosterone administration after gonadectomy reduces ACTH and corticosterone production (Seale *et al.*, 2004). In contrast, removal of the ovaries results in lower baseline corticosterone levels and attenuated corticosterone secretory responses to stress, compared with intact females; an effect that can be normalised with estrogen replacement (Seale *et al.*, 2004; Babb *et al.*, 2013). Hormonal changes during the estrous cycle also contribute to the activational effects of steroids, with basal concentrations of ACTH and corticosterone highest at proestrus, when estradiol levels peak (Atkinson & Waddell, 1997). Progesterone also plays a modulatory role in regulating the HPA axis in females, but in contrast to estradiol, progesterone has a seemingly inhibitory action, as it prevents the enhancing effects of estrogen on stress-induced ACTH secretion in rats (Viau & Meaney, 1991; Roy *et al.*, 1999). These gonadal steroids can thus be considered mediators of allostasis, where changes in their secretion in response to stress, can in turn shape HPA axis stress responses.

Although the organisational-activational theory for sexual differentiation has been widely applied, there are several unanswered and unaddressed questions surrounding this theory (Arnold, 2009; Lenz *et al.*, 2012; McCarthy *et al.*, 2017). Of particular relevance here is that although a wealth of studies support a critical role for gonadal steroids in mediating sex differences in stress responses, the underlying molecular mechanisms of steroid action remain unclear. Hence, next we explore the evidence for neuroactive steroids in mediating the effects of gonadal steroids on the HPA axis and the resultant sex differences in responses to stress.

NEUROACTIVE STEROIDS

Neuroactive steroids are active metabolites of classical steroid hormones that have rapid membrane actions on neuronal excitability. The brain expresses the enzymes necessary for neurosteroidogenesis (Compagnone and Mellon, 2000; Furukawa et al., 1998) in neurones and glia and can produce neurosteroids *de novo* from cholesterol (Baulieu, 1991;(Compagnone & Mellon, 2000). Indeed, neurosteroids continue to be detected in the brain after combined adrenalectomy and gonadectomy (Corpechot *et al.*, 1993). The first step in neurosteroidogenesis involves the conversion of cholesterol to pregnenolone which is catalysed by steroidogenic acute regulatory protein (StAR) and p450 side-chain cleavage enzyme (p450scc) (Fig. 3). Pregnenolone is subsequently converted into a range of downstream progestogens, androgens, estrogens and corticosteroids (Fig. 3). In addition, the brain can metabolise circulating steroid precursors produced in the periphery into neuroactive steroids (Compagnone & Mellon, 2000).

Gonadal sex steroids can be converted into neuroactive metabolites in the brain

Gonadal sex steroids can be metabolised by steroidogenic enzymes such as 5α-reductase, 3α- and 3β-hydroxysteroid dehydrogenase (3α-HSD and 3β-HSD) in the brain (Compagnone & Mellon, 2000). For example, progesterone can be metabolised by 5α-reductase into dihydroprogesterone (DHP), which in turn is converted into allopregnanolone by 3α-HSD (Fig. 3). Similarly, testosterone can be converted to dihydrotestosterone (DHT) by 5α-reductase and then into either 3α- or 3β-androstanediol (3α-diol and 3β-diol) through the actions of 3α-HSD and 3β-HSD, respectively (Fig. 3). The organisational actions of testosterone during the fetal surge are attributed to its conversion in the brain to estradiol (by aromatase), while its activational actions in adulthood are evidently a result of testosterone's downstream conversion to DHT and androstanediol (Arnold, 2009).

Neuroactive steroid signalling in the brain

Classical genomic actions of steroids are mediated by binding to steroid receptors such as GR, MR, progesterone receptors (PR), androgen receptors (AR), and estrogen receptors (ER), which are all ligand-dependent transcription factors that bind to steroid responsive elements to

enhance or repress gene transcription (Beato, 1989)(Fig. 4). However, it has become clear over the past 20 years or so that steroids do not only act via classic steroid receptor actions, as many steroid-mediated cellular responses occur within minutes, which is generally considered too short a time frame for genomic effects to occur (Makara & Haller, 2001; Simoncini & Genazzani, 2003).

Indeed, steroids can also exert rapid, neurotransmitter-like actions — they can bind to steroid receptors that do not exert direct genomic effects (e.g. those that are membrane bound, or those that are located in the cytosol but signal through other receptors), or bind to receptors that are not classical steroid receptors (e.g. transmembrane ion-channel receptors) (Cato *et al.*, 2002). These downstream effects that are rapid and do not directly affect gene expression, are defined as non-genomic actions of steroids (Lösel & Wehling, 2003). There are a wide range of receptors that steroids can act upon to exert non-genomic actions (for review see (Rupprecht, 2003); Fig. 4), but perhaps the most well characterised are the rapid allosteric actions on the $GABA_A$ receptors, resulting in a potentiation of GABA action (Groeneweg *et al.*, 2011; Gunn *et al.*, 2011). 3α,5α-reduced metabolites of DOC, progesterone, and testosterone (i.e. THDOC, allopregnanolone and 3α-diol, respectively) bind to a site distinct from the GABA binding region, opening the ion channel and permitting the enhanced influx of Cl^- ions by increasing both the channel opening frequency and the channel opening duration, which leads to sustained hyperpolarisation of the neurone and increased inhibition of neural activity (Majewska *et al.*, 1986; Morrow *et al.*, 1987; Lambert *et al.*, 2001; Hosie *et al.*, 2006; Akk *et al.*, 2007). Through these $GABA_A$-mediated actions, allopregnanolone and THDOC are able to temper HPA axis activity (Gunn *et al.*, 2015).

Neuroactive steroids modulate stress responses

Neuroactive metabolites of sex steroids play important roles in modulating HPA axis function. As mentioned above, allopregnanolone and THDOC facilitate termination of the stress response (Purdy *et al.*, 1991). Moreover, allopregnanolone and THDOC attenuate stress-induced HPA axis activity in male and female rats (Owens *et al.*, 1992; Patchev *et al.*, 1996; Brunton *et al.*, 2009) and have anxiolytic actions (Patchev *et al.*, 1994; Dubrovsky, 2005; Schule *et al.*, 2011). Importantly, the effects of testosterone in dampening stress-evoked HPA axis activity can be blocked by central administration of the 5α-reductase inhibitor, finasteride,

indicating that 5α-reductase activity in the brain is crucial for testosterone's actions (Handa *et al.*, 2009). Indeed, the inhibitory effect of testosterone on HPA axis activity is actually mediated by its metabolite 3β-diol acting via central estrogen receptor beta (ERβ) (Lund *et al.*, 2006; Foradori *et al.*, 2008; Handa *et al.*, 2009), whereas the anti-anxiety effects of 3α-diol act via a $GABA_A$-mediated pathway (Frye *et al.*, 1996; Edinger & Frye, 2005).

Sex differences in neuroactive steroid levels and action in the brain

In the adult rat brain, there are sex- and region-dependent differences in basal concentrations of neuroactive steroids (Caruso *et al.*, 2010b; Caruso *et al.*, 2013; Giatti *et al.*, 2019b). In general, levels of ovarian hormones such as progesterone and its metabolites are greater in the brains of females, whilst testicular hormones such as testosterone and its metabolites, are higher in males (Table 1). Patterns of the steroidogenic enzymes are also expressed in a sex-specific manner in the rat brain. For example, in the cerebellum, gene expression for StAR (*Stard1*) and 3α-HSD (*Akr1c4*) are significantly higher in females, whereas 5α-reductase expression is higher in males (Giatti *et al.*, 2019a). As these sex differences have recently been reviewed (Giatti *et al.*, 2019b), here we will focus on sex differences in neuroactive steroid levels in response to stress.

Sex differences in neuroactive steroid levels under physiological stress conditions

Rodent studies

Acute stress increases the central expression of 5α-reductase in a region dependent manner in male rats (Sanchez *et al.*, 2008a), however no studies have directly compared the impact of stress on steroidogenic enzyme expression in the brains of males and females. However, acute stress increases the levels of several neuroactive steroids in the brain (Purdy *et al.*, 1991; Vallée *et al.*, 2000), and the increase is more pronounced in females than in males (Sze *et al.*, 2018). Following acute swim stress, circulating corticosterone, DOC and progesterone concentrations are increased in both sexes; however, the levels are significantly greater in females compared to males (Sze *et al.*, 2018). In the brain, this sex difference is also observed in the majority of brain regions for DOC and progesterone, but not for corticosterone (Sze *et al.*, 2018). Notably, while stress-induced increases in allopregnanolone are observed in both sexes in the

prefrontal cortex, the effects are more marked in females, and in other regions such as the amygdala and brainstem, stress increases allopregnanolone concentrations only in female rats (Sze *et al.*, 2018). Many of the steroids are evidently produced *de novo* in the brain in response to stress, as central concentrations of pregnenolone, DHP and dihydrodeoxycorticosterone (DHDOC) increase, despite no corresponding change in the circulation (Sze *et al.*, 2018). Sex differences in neuroactive steroid levels in the brain after stress may be attributed to the differential availabilities of precursor steroids (e.g. progesterone), differential expression of steroidogenic enzymes (Giatti *et al.*, 2019a), or may constitute a downstream effect of sex differences in central CRH production and/or action (Torres *et al.*, 2001).

Furthermore, it is unclear whether these sex differences in central neuroactive steroid levels following stress play a role in differential HPA axis responses to stress. Increased stress-induced levels of allopregnanolone in the brain in females may be expected to result in a more rapid termination of the stress response, however this does not appear to be the case, as HPA axis stress responses resolve more slowly in females than in males (Burgess & Handa, 1992). This may be a result of progesterone acting as an antagonist at GRs (Svec, 1988), impeding glucocorticoid negative feedback. Nevertheless, greater allopregnanolone production in females following stress (Sze *et al.*, 2018) may represent a compensatory inhibitory signalling mechanism to aid termination of the stress response.

On the other hand, given limbic brain structures modulate the activity of the HPA axis via neural projections to GABAergic relay sites (e.g. in the peri-PVN and BNST)(Cullinan *et al.*, 2008), it is possible that sex differences in $GABA_A$ receptor-active neuroactive steroids (e.g. allopregnanolone) may lead to sex-dependent modulation of GABAergic signalling pathways upstream of the mpPVN. If this were the case it could be that in females there is greater disinhibition of CRH neurones in the mpPVN; an effect which would be expected to contribute to sex differences in HPA axis responses to stress.

CHRONIC STRESS AND HPA AXIS DYSREGULATION

Until now we have focussed on the physiological HPA axis response of a healthy adult organism to a single, acute stressor. However, depending on the severity, frequency, duration of the stressor, and the period of life in which the stressor is experienced, the HPA axis

response may differ considerably. While repeated homotypic stress may lead to the habituation of stress responses, chronic stress may result in sensitisation of stress responses, resulting in glucocorticoid production that is of greater amplitude or frequency (Melia *et al.*, 1994; Gomez *et al.*, 1996; Ma & Lightman, 1998), leading to "allostatic load" (Diamond *et al.*, 1992; de Kloet *et al.*, 1998). Prolonged allostatic load may eventually lead to "allostatic overload", where the impacts of HPA axis dysregulation manifests as the failure of physiological systems and the onset of disease (Natelson *et al.*, 1988; Belda *et al.*, 2015; Kinlein *et al.*, 2015). A hyperactive HPA axis is associated with mood disorders such as anxiety and depression, while insufficient activation of the HPA axis is often reported in patients with post-traumatic stress disorder (PTSD) (Sherin & Nemeroff, 2011; Pitman *et al.*, 2012).

Increased glucocorticoid production may result from an increased drive towards the production of CRH and ACTH, or altered receptor sensitivity in the key regions regulating the HPA axis (e.g. CRH receptors in the anterior pituitary, ACTH receptors in the adrenal glands). HPA axis dysregulation can also manifest as prolonged secretion of glucocorticoids, which may stem from decreased feedback inhibition either by GR-mediated mechanisms, or other neurotransmitter systems (Levy & Tasker, 2012). For instance, chronic stress-induced hyper-reactivity of the HPA axis is associated with an imbalance between excitatory and inhibitory signals, where there is generally increased excitatory (Flak *et al.*, 2009) and decreased inhibitory neurotransmission (Verkuyl *et al.*, 2004). Prolonged stress may also induce neuroactive steroid tolerance at the $GABA_A$ receptor, leading to the inability to properly terminate the HPA axis response (Turkmen *et al.*, 2011). In support, the levels of the $GABA_A$-modulatory neuroactive steroids, such as allopregnanolone and THDOC, are decreased in the brain following chronic stress in male mice and rats (Serra *et al.*, 2000; Dong *et al.*, 2001), while *Srd5a* expression has also been reported to be decreased following chronic stress in male mice (Agis-Balboa *et al.*, 2007). In clinical studies, levels of endogenous 3α-reduced neuroactive steroids in plasma and cerebrospinal fluid (CSF) are decreased in patients with affective disorders, whereas elevated neuroactive steroid levels are associated with anti-depressant actions (Uzunova *et al.*, 2006; Schule *et al.*, 2014).

Sex differences in HPA axis function under pathophysiological stress conditions

As a result of the underlying differences in physiology between males and females, consequences of "allostatic load" may differ between the sexes. Therefore, changes in the brain and behaviour following chronic or severe stress may be different for males and females, even if the same stressor is applied (Goel *et al.*, 2014).

Rodent studies of chronic stress

Chronic stress alters HPA axis function, however, animal models of chronic stress have mainly used male rodents, and there is a relative paucity of information for females, or direct comparative studies between males and females (Heck & Handa, 2019). A few studies have reported that females display more pronounced HPA axis dysregulation following chronic stress, with greater increases in corticosterone secretion (Dalla *et al.*, 2005; Vieira *et al.*, 2018). However, more studies are necessary to fully understand sex differences in responses to chronic stress. For example, it is not known whether the reduction in THDOC and allopregnanolone following chronic stress reported in male rodents (Serra *et al.*, 2000; Dong *et al.*, 2001) also occurs in females, and if so whether this effect is more or less pronounced than in males. This is important to establish as loss of this regulatory neuroactive steroid action is proposed to underlie stress-related disorders (Schule *et al.*, 2014; Maguire, 2019), for which males and females display different susceptibilities (Altemus *et al.*, 2014).

Sex differences also exist in behaviour reminiscent of stress-related psychiatric disorders in humans (Kokras & Dalla, 2014). However, due to the inherent variability in behavioural testing, inconsistencies regarding the direction of sex differences are frequently reported (Kokras & Dalla, 2014). Nevertheless, male rodents appear to be more vulnerable to stress-induced alterations in behaviour, as anxiety-like behaviour tends to be enhanced by chronic stress to a greater extent in males than females (Guo *et al.*, 2004; Bowman *et al.*, 2009). The response to severe trauma is also sex-dependent in a rat model of PTSD, where trauma-exposed males show a hyporesponsive HPA axis, due to exaggerated negative feedback control mechanisms, whereas these findings are not observed in female rats (Pooley *et al.*, 2018).

Again, sex differences in responses to chronic stress likely depends on the nature of the stressor(s), as there appear to be sex-specific differences in the appraisal/perception of how stressful a particular experience is. For example, over-crowding has been shown to be more

stress-inducing for males, whereas social isolation is more stressful for females (Brown & Grunberg, 1995; Palanza, 2001; Ramos-Ortolaza *et al.*, 2017). Thus, resilience and vulnerabilities to chronic stress are complex and it is likely that sex-specific vulnerabilities arise from the inherently different strategies used to cope with the stressor, rather than an inability to cope with the stressor *per se* (Leuner *et al.*, 2004; Pooley *et al.*, 2018).

SEX DIFFERENCES IN THE INCIDENCE OF AFFECTIVE DISORDERS IN HUMANS

Epidemiological studies indicate that men and women display different risk profiles for stress-related diseases. For instance, women are more prone to developing anxiety, depression, post-traumatic stress disorder, panic and eating disorders; while men show more antisocial behaviour and substance abuse (Altemus *et al.*, 2014; Bangasser & Valentino, 2014). Women also report higher incidences of stress, which may be attributed to underlying differences in stress responsivity (Wellman *et al.*, 2018). In clinical studies, HPA axis dysregulation is strongly implicated in psychiatric disorders. Hyper-secretion of cortisol is linked with mood disorders such as major depression or bipolar disorder (Plotsky *et al.*, 1998); whereas hypoactivation of the HPA axis is commonly observed in PTSD (Daskalakis *et al.*, 2016). There are also sex differences in the interaction between stress-induced cortisol responses and mood disorders, where women with depression or anxiety disorders display an overall blunted response to the TSST; while in men, an exaggerated response is observed (Zorn *et al.*, 2017).

Neuroactive steroids and affective disorders

Deficits in 5α-reduced neuroactive steroids, such as allopregnanolone have been implicated to play a role in affective disorders. Depressed patients have lower concentrations of allopregnanolone in the plasma and CSF and allopregnanolone levels are negatively correlated with depression severity (Romeo *et al.*, 1998; Uzunova *et al.*, 1998; Nappi *et al.*, 2001). In support, blocking 5α-reductase with finasteride results in decreased levels of allopregnanolone and DHT in the CSF and is associated with the development of anxiety and depression symptoms in these men (Melcangi *et al.*, 2013).

In animal models of postpartum depression, disrupted GABA signalling following dramatic changes in the hormonal milieu has been implicated as a causative factor, but neuroactive

steroid levels have not been measured (Lonstein *et al.*, 2014). Nonetheless, a recent clinical trial has demonstrated therapeutic effects in women with postpartum depression with a synthetic allopregnanolone analogue (Kanes *et al.*, 2017), further supporting a role for disrupted neuroactive steroid generation and/or action in mood disorders.

Sex differences in neuroactive steroids in psychiatric disorders

While the involvement of neurosteroids in rodent models of depression are convincing (Maguire, 2019), a role for neuroactive steroids in mediating sex differences in the propensity for psychopathology has yet to be investigated. Studies in humans are also lacking. Post-mortem analysis of brains from male patients with PTSD, indicate lower levels of allopregnanolone in the medial prefrontal cortex compared to healthy control males; whereas in females increased levels of pregnenolone relative to control females are observed (Cruz *et al.*, 2019), which may indicate a deficiency in the conversion of pregnenolone into downstream neuroactive metabolites. Consistent with this, another study has demonstrated a decrease in the conversion of DHP into allopregnanolone in the plasma of patients with PTSD (Pineles *et al.*, 2018), suggesting 3α-HSD dysfunction.

Taken together the data support a role for both HPA axis dysfunction and deficits in neurosteroids in stress-related psychiatric disorders, however far more research is needed to establish whether neuroactive steroids, such as allopregnanolone, underlie the distinct sex differences in the incidence of stress-related psychiatric disorders between males and females.

CHANGES IN STEROIDS OVER THE LIFETIME INFLUENCE STRESS RESPONSES AND VICE VERSA

In addition to the robust sex differences in HPA axis activity induced by steroid actions, there are dynamic changes in steroid production over the course of the lifetime and as a result periods when the 'set-point' of the HPA axis is reset and the HPA axis is more or less sensitive to stressors (Brunton, 2010; Konkle & McCarthy, 2011; Gupta & Morley, 2014; Green & McCormick, 2016; Romeo *et al.*, 2016; Oyola & Handa, 2017). HPA axis activity varies with sex, reproductive status, age and disease, with the common link being differences in sex steroid levels. Next we will discuss how changes in the steroid milieu and stress exposure during early

life, puberty and pregnancy differentially affect HPA axis function and alter the risk for mood disorders.

Sex differences in peripheral and central steroid levels during development

There are well-established sex differences in the extent to which the brain is exposed to testosterone and estradiol during the perinatal period, which contributes to the "organisation" of sex differences in the brain. As mentioned above, the male fetal brain is exposed to high levels of testosterone from their own testis, which peak in late pregnancy (Ward & Weisz, 1984) and surge again shortly after birth (Rhoda *et al.*, 1984). Maternal stress disrupts this testosterone surge in male foetuses and is associated with abnormal sexual behaviour in adulthood (Ward & Weisz, 1980; Ward & Weisz, 1984). In contrast, testosterone is consistently low in the perinatal female rat (Lieberburg *et al.*, 1979). Estradiol and DHT content in the fetal brain show a similar pattern in males and females in late pregnancy, with the exception of the hypothalamus on embryonic day 21 where estradiol levels are greater in males than females, probably as a result of aromatase actions in the brain (Konkle & McCarthy, 2011). Plasma estradiol levels are similarly elevated in both male and female rats at birth, before declining rapidly, reaching a nadir on PND 4 (Konkle & McCarthy, 2011).

Allopregnanolone concentrations in the fetal brain are remarkably high in late pregnancy and 5α-reductase is strongly expressed in the developing rat and sheep brain (Martini *et al.*, 1996; Nguyen *et al.*, 2003; Westcott *et al.*, 2008). In the rat, central allopregnanolone concentrations in the fetal brain decline before birth, even though central 5α-reductase expression persists, probably as a result of declining progesterone (Poletti *et al.*, 1998; Grobin & Morrow, 2001). While brain levels of allopregnanolone have not been characterised in the fetus, sex differences begin to emerge on PND 25, where concentrations are approximately 2-fold greater in females than in males (Grobin & Morrow, 2001).

The HPA axis in early life

In rats, CRH and ACTH immunoreactive cells are first identified at around day 16 of gestation in the fetal PVN and anterior pituitary, respectively (Schwartzberg & Nakane, 1982; Daikoku *et al.*, 1984). ACTH and corticosterone are also detectable in fetal plasma on day 16 of pregnancy

(Boudouresque *et al.*, 1988), however hypothalamic control of the fetal HPA axis only develops from around day 18 (Eguchi *et al.*, 1973). The fetal HPA axis is capable of responding to maternal stress during late gestation (Ohkawa *et al.*, 1991; Williams *et al.*, 1999; Fujioka *et al.*, 2003), however there appears to be a sex difference with males exhibiting a significant increase in ACTH secretion in response to maternal stress from day 18, whereas in females this is not observed until day 20 (Ohkawa *et al.*, 1991). Maternal stress-induced increases in corticosterone in the fetal circulation are observed earlier, however this is likely of maternal origin (Ohkawa *et al.*, 1991). Whereas, given ACTH does not cross the placenta (Dupouy *et al.*, 1980), the source of ACTH in the fetal circulation is considered to be the fetal pituitary gland.

In the early postnatal period (from ca. PND 3-14 in rats), the HPA axis is less reactive to stress, the so-called 'stress hyporesponsive period' (SHRP), which is thought to minimise the risk of excessive glucocorticoids disrupting the normal development of glucocorticoid-sensitive brain regions (Sapolsky & Meaney, 1986). Hence, in contrast to adults, neonates display a relative inability to elicit an ACTH or corticosterone response to certain stressors (Sapolsky & Meaney, 1986; Levine, 2001). Mother-pup interactions and increased sensitivity to glucocorticoid inhibitory feedback, rather than insufficient CRH/AVP production by the mpPVN neurones are major contributing factors underlying the SHRP (Walker *et al.*, 1990; Dent *et al.*, 2000). While hippocampal GR expression is fairly low at birth, it increases significantly over the first two weeks of life, with the highest levels of expression peaking at PND12, which potentiates negative feedback inhibition of the HPA axis (Schmidt *et al.*, 2003). It has been suggested that increased neurosteroid biosynthesis may contribute to maintain stress hyporesponsiveness during infancy as high levels of *Akr1c4* expression are detected in the hippocampus on postnatal day 7, coinciding with the SHRP in the rat (Mitev *et al.*, 2003), though this remains to be thoroughly interrogated.

Impact of early life stress on HPA axis function

Stress experienced in early life, either prenatally or in the early postnatal period, is well established to have long term 'programming' effects on the brain and behaviour (Weinstock, 2007; Bale *et al.*, 2010; Maccari *et al.*, 2014). The HPA axis is especially vulnerable to the adverse effects of chronic stress exposure in early life, with augmented and/or protracted HPA axis responses typically observed in rodents exposed to early life stress (Fride *et al.*, 1986; Takahashi

& Kalin, 1991; Weinstock *et al.*, 1992; Henry *et al.*, 1994; McCormick *et al.*, 1995; Barbazanges *et al.*, 1996; Morley-Fletcher *et al.*, 2003b; Bosch *et al.*, 2007; Mueller & Bale, 2008; Fan *et al.*, 2009; Brunton & Russell, 2010b). Furthermore, the neonatal SHRP is absent in pups born to stressed mothers (Henry *et al.*, 1994) and adult offspring fail to habituate to repeated exposure to the same stressor (Fride *et al.*, 1986). Likewise, heightened stress-related behaviours, such as anxiety- and depressive-like behaviours and altered stress-coping are frequently reported in animals exposed to early life stress (Fride & Weinstock, 1988; Morley-Fletcher *et al.*, 2003a; Lee *et al.*, 2007; Mueller & Bale, 2008; Fan *et al.*, 2009; Brunton & Russell, 2010b; Raineki *et al.*, 2012), with comparable findings in humans (Heim & Nemeroff, 2001; Van den Bergh *et al.*, 2008; Pawlby *et al.*, 2009).

Sex differences in early life stress effects

For both the HPA axis and stress-related behaviours, sex differences are frequently observed in the resultant phenotypes induced by early life stress (Weinstock, 2007); for example, males often appear more vulnerable to the behavioural effects of prenatal stress than females. Exposure of rats to repeated restraint or social stress during the last week of pregnancy both result in an anxious phenotype in the adult male, but not in the adult female offspring (Zuena *et al.*, 2008; Brunton & Russell, 2010b; Iturra-Mena *et al.*, 2018); with similar sex-specific effects on anxiety-like behaviour reported in the male offspring of guinea pigs exposed to chronic stress during the second half of pregnancy (Emack *et al.*, 2008). Sex differences in anxiety-like behaviour may be a result of the anxiolytic actions of estradiol in females (Walf & Frye, 2007). Sex-dependent effects of prenatal stress exposure in early gestation on offspring behaviour have also been reported in mice (Mueller & Bale, 2008). Male, but not female prenatally stressed offspring show less active stress coping behaviours in the forced swim and tail suspension test, and anhedonia in the sucrose preference test; behaviours which are considered to be indicative of a depressive-like phenotype (Mueller & Bale, 2008). However, these sex differences seem to be dependent on the species, stress paradigm used, the timing of the stress exposure and the age of the animals at testing, as some studies report greater anxiety- and depressive-like behaviour in the offspring of both sexes (Fride & Weinstock, 1988; Butkevich *et al.*, 2019; Verstraeten *et al.*, 2019) or that females are more affected than males (Keshet & Weinstock, 1995; Richardson *et al.*, 2006; Sickmann *et al.*, 2015).

Relatively few studies have directly compared the HPA axis response to stress prenatally stressed male and female offspring in the same experiment. Under basal conditions, both male and female prenatally stressed rats secrete significantly higher levels of corticosterone at the end of the light period (Koehl *et al.*, 1999). In terms of HPA axis responses to acute stress in prenatally stressed offspring, some studies report responses that are greater in amplitude and/or prolonged in both sexes (Smith *et al.*, 2004; Brunton & Russell, 2010b); whereas others using different stress paradigms find sex differences where females are more severely affected than males (Brunton & Russell, 2010b) or only females display HPA axis hyperactivity (Weinstock *et al.*, 1992; McCormick *et al.*, 1995; Bakker *et al.*, 1998; Richardson *et al.*, 2006); again highlighting that effects are dependent on multiple factors. Some of these sex differences are likely attributable to sex steroids, as males exposed to neonatal stress by maternal deprivation display an anxious phenotype irrespective of their adult gonadal status, whereas the anxiogenic effects of early life stress in females only become apparent after ovariectomy (Mitev *et al.*, 2003), again supporting an anxiolytic action of estradiol in females (Walf & Frye, 2007; Walf *et al.*, 2008).

In the case of prenatal stress, placental gene expression may also contribute to sex differences in adult phenotypes. During pregnancy, placental 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) converts maternal glucocorticoids into their inert metabolites to minimise transplacental transfer of glucocorticoids and protect the fetus from glucocorticoid over-exposure (Benediktsson *et al.*, 1997). However, there are sex differences in the effect of maternal stress on the activity of this enzyme. For example, in mice, stress exposure in mid-gestation increases placental 11β-HSD2 (*Hsd11b2*) gene expression in both sexes (though the increase is delayed in females), but increased 11β-HSD2 activity is only observed in males; which leads to increased glucocorticoid exposure in the female foetuses (Wieczorek *et al.*, 2019). We have also recently found similar sex-specific effects on *Hsd11b2* gene expression in rats exposed to social stress in late pregnancy (Sze, Fernandes & Brunton, unpublished observations). Given exposure to excessive levels of glucocorticoids during pregnancy has adverse effects on the developing fetal brain and results in HPA axis dysfunction (Welberg *et al.*, 2000; Welberg *et al.*, 2001; Banjanin *et al.*, 2004; Dunn *et al.*, 2010), sex-specific differences in placental 11β-HSD2 expression and the resultant differences in fetal glucocorticoid

exposure, may contribute to sex differences in adult behaviours and HPA axis responsivity following prenatal stress.

Furthermore, stress in early pregnancy in mice results is a sex-specific effect on the expression of genes that support the fetal transport of critical growth factors and nutrients, such as peroxisome proliferator-activated receptor-α (PPARα) and insulin-like growth factor binding protein-1 (IGFBP-1). While prenatal stress increases PPARα (*Ppara*) and IGFBP-1 (*Igfbp1)* gene expression in male placentas, the opposite effect is observed in female placentas (Mueller & Bale, 2008). Given PPARα directly increases the expression of *Igfbp1* (Degenhardt *et al.*, 2006) and increases in placental IGFBP-1 result in a decrease in available growth factors (as IGFBP-1 blocks insulin-like growth factors binding to the insulin receptor), differential responses of this gene to stress may contribute to sex differences in fetal programming.

Mechanisms involved in early life programming

A plethora of studies have sought to understand the central mechanisms underpinning early life programming of the stress system and behaviour. Evidence for increased excitatory drive to the CRH neurones in the PVN, impaired glucocorticoid negative feedback control, altered glutamatergic signalling in the prefrontal cortex, amygdala and hippocampus and altered signalling via central CRH receptors have been reported and reviewed elsewhere (Henry *et al.*, 1994; Mueller & Bale, 2008; Zuena *et al.*, 2008; Fan *et al.*, 2009; Brunton & Russell, 2010b; Brunton *et al.*, 2011; Laloux *et al.*, 2012; Marrocco *et al.*, 2012; Gunn *et al.*, 2013; Maccari *et al.*, 2014; Marrocco *et al.*, 2014; Buonaguro *et al.*, 2019). Here, we focus on a potential role for altered neurosteroidogenesis in mediating some of the impacts of early life stress and the underlying sex differences.

Early life stress and neurosteroidogenesis

Maternal exposure to prolonged stress or high levels of exogenous glucocorticoids during pregnancy appears to reduce the neurosteroidogenic capacity of the fetal brain in rodents, much like chronic stress in adulthood does (discussed above). In rats, maternal stress in late pregnancy reduces 5α-reductase activity in the brain of male foetuses (females were not studied)(Ordyan & Pivina, 2005) and reduces circulating allopregnanolone levels in the dams;

which predicts lower allopregnanolone generation in both the male and female offspring's brain in later life (Paris & Frye, 2011b). In guinea pigs, treatment with the synthetic glucocorticoid, betamethasone in late pregnancy, leads to reduced gene expression for 5α-reductase type 2 (*Srd5a2*) in the brain of male, but not female foetuses (McKendry *et al.*, 2010); whereas 5α-reductase type 1 (*Srd5a*1) mRNA expression in the brain is increased by betamethasone treatment in female foetuses, but not in males (McKendry *et al.*, 2010). Preterm birth (which leads to a premature decline in allopregnanolone levels and increased glucocorticoid secretion in the neonate) also leads to a reduction in the expression of key $GABA_A$ receptor subunits that normally increase neurosteroid sensitivity in both the male and female neonates (Shaw *et al.*, 2015; Hirst *et al.*, 2016).

Reduced 5α-reductase activity in the fetal brain following maternal stress seems to persist into later life, as both the male and female juvenile offspring of rats exposed to chronic stress during pregnancy show reduced conversion of progesterone into its 5α-reduced metabolite, DHP in the medial prefrontal cortex (Paris & Frye, 2011b; Paris & Frye, 2011a); whereas in the diencephalon, DHP is increased in prenatally stressed females, but decreased in prenatally stressed males (Paris & Frye, 2011a). In adulthood, the male offspring (again females were not studied) of rat dams exposed to repeated stress in late gestation, have lower hippocampal levels of the 5α-reduced metabolites of testosterone, DHT and 3α-diol and this is associated with greater corticosterone responses to stress and heightened anxiety behaviour (Walf & Frye, 2012). Moreover, we have shown sex-dependent effects of maternal social stress in late gestation on 5α-reductase gene expression in the hypothalamus and medulla of the adult offspring: expression in the NTS is reduced in both male and female prenatally stressed rats, whereas in the PVN, *Srd5a1* expression is reduced only in the male offspring (Brunton *et al.*, 2015). The mechanism underlying the sex-dependent effects of prenatal stress on central 5α-reductase expression, activity, and consequently neurosteroid production is not clear, though differences in circulating testosterone may contribute (Torres & Ortega, 2006; Purves-Tyson *et al.*, 2012; Sanchez *et al.*, 2012). Indeed, 5α-reductase activity in the liver is programmed during the first week of life by testicular androgens, hence testectomy stimulates, while testosterone administration inhibits hepatic 5α-reductase activity (Gustafsson & Stenberg, 1974). Consistent with this theory, prenatally stressed male rats with increased plasma testosterone

concentrations (Brunton *et al.*, 2015) also exhibit lower hepatic 5α-reductase gene expression (Brunton *et al.*, 2013).

Stress exposure in early postnatal life has similar effects to prenatal stress on 5α-reductase activity, however only a few studies have investigated whether reduced 5α-reductase leads to reduced neuroactive steroid concentrations in the brain of animals stressed in early life. Male juveniles exposed to chronic psychosocial stress from weaning, exhibit significantly lower protein expression of both 5α-reductase isoforms in the medial prefrontal cortex and nucleus accumbens, concomitant with reduced allopregnanolone and THDOC content in the frontal cortex (Bortolato *et al.*, 2011); and reduced DHP synthesis is observed in the brains of male and female neonates exposed to stress via daily separation from the dam and littermates during the first 9 days of life (Kehoe *et al.*, 2000). Moreover, social isolation in juvenile male rats at weaning results in markedly reduced levels of allopregnanolone and THDOC in the blood and brain and reduced central $GABA_A$ receptor function, which is associated with increased anxiety-like behaviour, increased sensitivity of the pituitary corticotrophs to CRH and impaired negative feedback control of the HPA axis (Serra *et al.*, 2000; Serra *et al.*, 2005). Similar findings have been reported for central allopregnanolone and THDOC levels in female rats reared in isolation from weaning, which are also more anxious compared with socially-reared rats (Pisu *et al.*, 2013). More recently, the same group have made direct comparisons between male and female rats reared in social isolation in early life and demonstrated that the chronic stress associated with social isolation at weaning, reduces basal levels of circulating and central allopregnanolone to a similar extent in both sexes (Pisu *et al.*, 2016). Glucocorticoid feedback inhibition of the HPA axis is also impaired in both sexes, and males and females display markedly greater corticosterone responses to acute stress compared with group-housed animals. However this effect is more pronounced in male rats than in females subjected to social isolation; and only socially isolated males display depressive-like behaviour (Pisu *et al.*, 2016).

Stress in early life also disrupts neuroactive steroid action in the brain. In mice, early life stress induced by fragmented maternal care results in increased excitatory afferent drive to the parvocellular CRH neurones in the PVN and up-regulated *Crh* gene expression (Gunn *et al.*, 2013). Intriguingly, while allopregnanolone is effective in suppressing the neuronal firing rate

of PVN CRH neurones in control mice, this action is compromised by prior early life stress exposure (Gunn *et al.*, 2013). This insensitivity to allopregnanolone evidently results from increased glutamatergic drive to the CRH neurones, rather than disrupted GABAergic signalling (Gunn *et al.*, 2013), however it is not known whether any sex differences exist in the degree of allopregnanolone insensitivity.

A role for neuroactive steroids in counteracting early life stress effects

Given the findings that early life stress disrupts neurosteroidogenesis, in particular 5α-reduced steroid production, it has been hypothesised that this may underlie some of the adverse effects of early life stress; such as HPA axis dysregulation and increased anxiety-like behaviour. Therefore studies have investigated whether neuroactive steroid treatment can prevent or reverse the programming effects of perinatal stress exposure. Administration of allopregnanolone to pregnant rats in conjunction with stress exposure, prevents the increase in anxiety-behaviour typically induced by prenatal stress in the neonatal and adult offspring (Zimmerberg & Blaskey, 1998) and reduces ultrasonic vocalizations in rat pups induced by maternal separation (Zimmerberg *et al.*, 1994; Vivian *et al.*, 1997). Similarly, THDOC administered during extended periods of maternal deprivation in the early postnatal period, is effective in preventing the neuroendocrine and behavioural consequences of neonatal stress, including exaggerated HPA axis responses to stress, impaired glucocorticoid feedback control of the HPA axis and increased anxiety behaviour in males (females were not studied) (Patchev *et al.*, 1997); while similar treatment with allopregnanolone counteracts these adverse effects in both sexes (Mitev *et al.*, 2003).

In adulthood, short-term allopregnanolone administration is effective in attenuating the hyperactive HPA axis responses usually observed in prenatally stressed females, but not in their male littermates (Brunton *et al.*, 2015). However, treatment with the testosterone metabolite, 3β-diol, does normalise enhanced HPA axis responses to stress in the adult prenatally stressed male offspring (Brunton *et al.*, 2015). Furthermore, up-regulating *Srd5a1* and *Akr1c4* gene expression in the brainstem using adenoviral vectors also prevents hyperactive HPA axis responses to acute stress in prenatally stressed rats (Brunton *et al.*, 2015), presumably by enhancing local neurosteroid synthesis, though this remains to be tested.

Taken together these data indicate that neuroactive steroids, especially those that act as $GABA_A$ receptor agonists, may protect the developing brain from the adverse effects of perinatal stress or reverse the neuroendocrine effects when administered in later life. The mechanisms involved are not clear, but $GABA_A$ receptors are likely targets for allopregnanolone and THDOC actions, given their affinity for these receptors and their potent stress-suppressing and anxiolytic actions through potentiating GABA signalling (Patchev *et al.*, 1994; Patchev *et al.*, 1996). Indeed, there is evidence that the suppressive effect of allopregnanolone on ultrasonic vocalisations induced by maternal separation is mediated by $GABA_A$ receptors (Vivian *et al.*, 1997). In contrast, 3β-diol modulates HPA axis responses and anxiety-like behaviour through actions on estrogen receptor-β in the PVN (Lund *et al.*, 2006; Weiser *et al.*, 2009); though it has yet to be established if this is how it exerts its effects in prenatally stressed rats (Brunton *et al.*, 2015).

Sex differences in the sensitivity of central $GABA_A$ receptors to neurosteroids have been reported, with females exhibiting greater sensitivity to the anti-seizure and neuroprotective effects of allopregnanolone (Kelley *et al.*, 2011; Reddy *et al.*, 2019). Whether this is also the case for $GABA_A$-mediated actions on HPA axis activity is not known, but warrants investigation.

Sex-specific effects of paternal stress on the offspring

In recent years, some researchers have begun to investigate the impact of chronic paternal stress around the time of conception on offspring HPA axis function. The limited evidence indicates paternal stress also results in dysregulated HPA axis responses to stress in the offspring, though the direction of the change seems dependent on the stress paradigm used. One study in mice reported, increased basal plasma concentrations of corticosterone in the male, but not female offspring (Dietz *et al.*, 2011); whereas another reported elevated baseline corticosterone levels in the offspring of both sexes, concomitant with reduced hippocampal GR expression (Niknazar *et al.*, 2017). In both studies, the male and female offspring of stressed fathers exhibited heightened anxiety-like behaviours (Dietz *et al.*, 2011; Niknazar *et al.*, 2017). In contrast to the effect of maternal stress, the offspring of stressed fathers appear to display attenuated HPA axis responses to acute stress exposure in later life (Rodgers *et al.*, 2013; Rodgers *et al.*, 2015), which may result from epigenetic reprogramming of the sperm (Rodgers *et al.*, 2015). However to date, and to the best of our knowledge, no studies have investigated

a role for altered neurosteroidogenesis in mediating the impact or the downstream consequences of paternal stress on the offspring.

Sex differences in HPA axis function at puberty

Puberty is accompanied by dynamic changes in circulating adrenal and gonadal steroids in males and females, which contribute to the organisation and maturation of the HPA axis (Viau *et al.*, 2005). Basal concentrations of circulating testosterone are significantly greater in post-pubertal males compared with pre-pubertal males, while in females, plasma estradiol, progesterone and corticosterone levels are all significantly greater after puberty (Viau *et al.*, 2005). It is unsurprising therefore, that these changes in sex and adrenal steroids at puberty are associated with altered HPA axis responsivity. For example, compared with post-pubertal males, pre-pubertal males display greater ACTH and corticosterone responses to acute stress (Vazquez & Akil, 1993) and stress-induced Fos induction and *Avp* gene transcription in the mpPVN is significantly lower after puberty (Viau *et al.*, 2005). This shift in HPA responsiveness in males at puberty corresponds with increased plasma testosterone levels (Romeo *et al.*, 2004), which inhibits HPA axis function (Viau *et al.*, 2003; Lund *et al.*, 2004). In contrast, stress-induced increases in ACTH and corticosterone secretion are greater in post-pubertal in females than in pre-pubertal females, despite similar levels of stress-induced Fos and *Avp* gene transcription in the mpPVN (Viau *et al.*, 2005), further supporting the idea that there are distinct sex differences in the regulation of the HPA axis at puberty.

Heightened stress reactivity around puberty may put adolescents at higher risk for psychopathology, and explain the increasing incidences of psychiatric disorders that arise during adolescence (Gunnar & Quevedo, 2007; Paus *et al.*, 2008). Indeed, sex differences in the risk for mood disorders rise sharply after puberty. For example, after puberty, incidences of major depression are two times higher in females compared to males and remain so until after menopause (Altemus *et al.*, 2014). The onset of puberty is also associated with increases in anxiety disorders, which are twice as likely to occur in girls as in boys (Hayward & Sanborn, 2002). In female mice, hippocampal allopregnanolone concentrations decline by around 50% at the onset of puberty (Shen *et al.*, 2007). However in contrast to its robust anxiolytic action in adults, allopregnanolone paradoxically increases anxiety-like behaviour in pubertal female mice (Shen *et al.*, 2007), which may be a result of allopregnanolone withdrawal as similar

findings are observed in a rodent model of premenstrual dysphoric disorder, where increases in allopregnanolone levels also exert anxiogenic effects (Smith *et al.*, 2006).

Pregnancy

Steroid milieu of pregnancy

One period during a female's life when steroid production is dramatically altered is during pregnancy. Circulating levels of the female sex steroids, estradiol and progesterone are considerably increased in pregnancy (Fig. 5) (Brunton & Russell, 2010a; Brunton *et al.*, 2014) providing a unique model in which to study the impact of these steroids (and their metabolites) on HPA axis function.

In the periphery, estradiol and progesterone act on the uterus to ensure optimal conditions for maintenance of pregnancy. In the rat, progesterone secretion by the corpus luteum steadily increases, peaking around day 15 and remaining elevated until ca. 2 days before term when there is a sharp decline in circulating progesterone concentrations (Fig. 5) (Brunton & Russell, 2010a). Estradiol secretion increases on day 4 of pregnancy, which coincides with implantation, then declines and fluctuates at intermediate levels until increasing towards the end of pregnancy, reaching a maximum on day 21 of gestation (Fig. 5) (Shaikh, 1971; Brunton & Russell, 2010a). In addition, to actions on the uterus to sustain the pregnancy, estradiol and progesterone also act in the brain, for example in the mPOA, to prime the neural circuitry necessary for the expression of appropriate maternal care after parturition (Bridges, 1984; Numan, 2007).

Concomitant with increased concentrations of progesterone in the brain and circulation in late pregnancy, is an increase in central and peripheral levels of its 5α-reduced metabolite, allopregnanolone (Concas *et al.*, 1998). However, unlike progesterone, allopregnanolone in the cerebral cortex does not reach maximal levels until later in pregnancy, at around day 19 (Fig. 5) (Concas *et al.*, 1998). Similarly, concentrations of another neuroactive steroid metabolite, THDOC is also markedly increased in the blood and brain in late pregnancy (Fig. 5) (Concas *et al.*, 1998). Consistent with these findings, gene expression and activity of the enzyme, 5α-reductase is increased in the hypothalamus and medulla in late pregnancy (Brunton *et al.*,

2009), as are concentrations of the steroid precursor, pregnenolone (Concas *et al.*, 1998), suggesting local neuroactive steroid generation may play an important role in pregnancy. The mechanism through which central 5α-reductase and 3α-HSD expression are regulated in pregnancy are not clear. The available evidence does not support a role for either estradiol or progesterone in regulating *Srd5a* gene expression in the brain, however increased levels of prolactin in pregnancy may play a role (Sanchez *et al.*, 2008b). On the other hand, estradiol does appear to regulate *Akr1c4* expression in the brain (Bertics *et al.*, 1987; Mitev *et al.*, 2003).

The maternal HPA axis in pregnancy

In early pregnancy in the rat, ACTH and corticosterone secretion at the circadian nadir are similar to those observed in non-pregnant rats. However, there is a marked change in the circadian rhythm of ACTH and corticosterone secretion in pregnancy. By day 2 of pregnancy the diurnal peak in ACTH secretion is suppressed by around one third of pre-pregnancy levels and remains so for the remainder of gestation. Peak corticosterone secretion is also similarly reduced by day 2 of pregnancy, however after day 10 both trough and peak corticosterone concentrations progressively increase, reaching a maximum on day 22. It is likely that increased corticosterone secretion, in the absence of an increase in ACTH secretion is the result of the adrenal gland becoming more sensitive to ACTH as a result of estradiol actions (Viau & Meaney, 1991). Similar observations have been made in pregnant women where cortisol levels are lower in early pregnancy before increasing in late pregnancy and it is thought that reduced glucocorticoid levels in early pregnancy may facilitate implantation (Allolio *et al.*, 1990; Nepomnaschy *et al.*, 2015).

In the first half of pregnancy, HPA axis responses to acute physical or psychological stressors are similar to those of non-pregnant females, however in late pregnancy the HPA axis becomes hyporesponsive to acute stress exposure (for review see (Brunton *et al.*, 2008)). This is indicated by reduced or even a complete absence of an ACTH and/or corticosterone secretory response to an array of both physical, and psychological stressors, as well as for stressors containing both psychological and physical elements (da Costa *et al.*, 1996; Douglas *et al.*, 1998; Neumann *et al.*, 1998; Brunton & Russell, 2003; Brunton *et al.*, 2005; Brunton *et al.*, 2006; Brunton & Russell, 2008a; Windle *et al.*, 2010). This has been demonstrated most extensively in the rat, but suppressed HPA axis responses to acute stress are also reported in late pregnant mice

(Douglas *et al.*, 2003) and women (Schulte *et al.*, 1990; Hartikainen-Sorri *et al.*, 1991; Kammerer *et al.*, 2002). Reduced HPA axis activity in late pregnancy is a result of adaptations in the anterior pituitary (Neumann *et al.*, 1998), reduced excitatory drive from limbic brain regions and the brainstem to the parvocellular CRH and AVP neurones in the PVN (da Costa *et al.*, 1996; Brunton *et al.*, 2005; Douglas *et al.*, 2005), reduced secretion of ACTH secretagogues by the PVN neurones (Ma *et al.*, 2005) and slow glucocorticoid negative mechanisms mediated by the hippocampus (Johnstone *et al.*, 2000).

Steroids and suppressed HPA axis responses in pregnancy

Despite increasing concentrations of female sex steroids coinciding with the manifestation of HPA axis hyporesponsiveness in pregnancy (Fig. 5), increased levels of estradiol and progesterone evidently do not play a role in the initiation or maintenance of suppressed HPA axis responses. Administration of estradiol or progesterone alone or in combination to mimic the steroid milieu of pregnancy in virgin rats is not sufficient to simulate the HPA axis hyporesponsiveness seen in late pregnancy (Douglas *et al.*, 2000; Brunton *et al.*, 2009).

In late pregnancy, it is in fact increased levels of allopregnanolone that act to restrain the HPA axis from responding to stress. Hence inhibiting allopregnanolone synthesis in late pregnant rats with the 5α-reductase inhibitor, finasteride, reinstates HPA axis responses to stress in late pregnancy (Brunton *et al.*, 2009). This treatment would also be expected to block THDOC production, however consistent with a role for allopregnanolone as the critical factor is the finding that allopregnanolone is able to supress HPA axis responses to stress in non-pregnant females (Brunton *et al.*, 2009). However the allopregnanolone precursors, progesterone and DHP are both ineffective in attenuating stress-induced ACTH secretion (Brunton *et al.*, 2009), highlighting the importance of central neurosteroidogenic enzymes in suppressed HPA axis responses to stress in pregnancy.

Allopregnanolone exerts its inhibitory effects on HPA axis stress responses by induction of a central endogenous opioid mechanism. In late pregnancy, endogenous opioids inhibit HPA axis responses to stress (Douglas *et al.*, 1998; Brunton *et al.*, 2005), evidently through actions in the PVN (Brunton *et al.*, 2005). The origin of these endogenous opioids is thought to be NTS neurones that project to the PVN. Indeed, gene expression for the opioid precursor,

proenkephalin-A (*Penk*) and the mu-opioid receptor (*Oprm1*) is significantly increased in brainstem NTS neurones in late pregnancy (Brunton *et al.*, 2005). Importantly, up-regulation of *Penk* is prevented in pregnant animals if allopregnanolone synthesis is blocked with finasteride, and this same treatment also restores stress-induced ACTH secretion in late pregnancy (Brunton *et al.*, 2009). Moreover, allopregnanolone administration to non-pregnant female rats induces a similar increase in opioid gene expression in the NTS and induces inhibitory opioid tone over the HPA axis, leading to attenuated HPA axis responses to stress (Brunton *et al.*, 2009). The mechanism by which allopregnanolone up-regulates endogenous opioid expression has yet to be elucidated, but it may involve an interaction with $GABA_A$ receptors as previously demonstrated for transcriptional regulation of CRH and AVP in the PVN (Bali & Kovacs, 2003).

Suppressed HPA axis responses to stress in pregnancy are anticipated to provide a protective mechanism to minimise fetal exposure to excessive levels of maternal glucocorticoids and thereby reduce the risk of adverse fetal programming, but also to support metabolic adaptations in pregnancy (Herrera, 2000). Nonetheless, it clear that this protective mechanism can be breached as exposure to chronic or repeated stress during pregnancy leads to adverse outcomes in the offspring, as described above. Few studies have focussed on maternal HPA axis responses to chronic stress in pregnancy. However, in rats, chronic social stress elicits a significant corticosterone response in both early and late pregnancy (Stefanski *et al.*, 2005; Brunton & Russell, 2010b) and chronic stress induces a significant increase in ACTH and corticosterone secretion in late pregnant rats (Takahashi *et al.*, 1998; Maghsoudi *et al.*, 2014). Chronic social stress in male rodents has been shown to reduce *Srd5a1* expression, 5α-reductase activity and allopregnanolone levels in the brain and it is thought that this leads to HPA axis hyperactivity (Serra *et al.*, 2000; Dong *et al.*, 2001; Matsumoto *et al.*, 2005; Agis-Balboa *et al.*, 2007; Evans *et al.*, 2012). It is not clear whether chronic social stress has a similar effect in late pregnancy, however if this were the case, it may explain why HPA axis responses to chronic stress are not effectively dampened in pregnancy and this may contribute to the programmed phenotypes observed in the offspring.

Pregnancy represents a period in the female's life when there is an increased allostatic load and changes in HPA axis responsiveness are not without cost. The sudden withdrawal of the

pregnancy hormones around birth may predispose the mother to postpartum depression (Brunton & Russell, 2008b). Women who have experienced postpartum depression show greater sensitivity to experimental estrogen and progesterone withdrawal (Bloch *et al.*, 2000) and the withdrawal of estradiol and progesterone induces depression-like behaviour in rat models of postpartum depression (Galea *et al.*, 2001; Stoffel & Craft, 2004). These data suggest that a loss of neurosteroid action in the brain may contribute to the manifestation of postpartum depression in vulnerable women. Indeed, as mentioned above, recent clinical trials have demonstrated that an allopregnanolone analogue (brexanolone) with positive allosteric actions at $GABA_A$ receptors is effective in the treatment of postpartum depression (Kanes *et al.*, 2017; Meltzer-Brody *et al.*, 2018). It is not known whether such treatments are also effective in treating other types of psychiatric disorders, such as major depressive disorder in men or non-pregnant women, however phase 2 clinical trials are currently underway (Hoffman *et al.*, 2019).

CONCLUDING REMARKS

Sex differences in HPA axis responsivity are well established, with females generally displaying greater reactivity than males. Differences in gonadal sex steroids and their neuroactive metabolites are largely responsible for sex differences in HPA axis responses to acute stress. This is further supported by the finding that changes in gonadal/neuroactive steroids that occur naturally at different times of life e.g. in the perinatal period, at puberty and during pregnancy, are reflected by concordant changes in the responsivity of the HPA axis.

Chronic stress or stress experienced during critical periods in development, such as the perinatal period, can lead to deficits in neuroactive steroid production and HPA axis dysfunction. HPA axis dysregulation is a common feature of stress-related psychiatric disorders and reduced levels of neuroactive steroids are implicated in altered emotionality in animal models and affective disorders in humans. A causative link between these two factors has yet to be fully established, however the findings from rodent early life stress models where neuroactive steroids can prevent or reverse dysregulated HPA axis function and normalise anxious behaviours, suggest that disruptions in neurosteroidogenesis underpin HPA axis dysregulation, rather than the opposite scenario.

Women are far more likely to be affected by mood disorders, however the evidence described in this review only indirectly supports a potential role for neuroactive steroids in the sex bias in stress-related psychopathologies. Progress in this area has been hampered by the apparent reluctance of many researchers to study females and the paucity of direct comparative studies between males and females. This is problematic as findings based on only one sex may lead to inaccurate conclusions and limit the effectiveness of any therapeutic interventions in the sex that is neglected (typically females). For example, given the marked sex differences in steroid production and hence stress axis regulation, therapeutics targeting steroid signalling pathways to treat stress-related psychiatric disorders may differ in efficacy between males and females. With this in mind there is an obvious need for future research to take into account both sexes, only then may we fully understand the factors that are crucial in accounting for sex differences in the propensity to develop affective disorders.

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AUTHOR CONTRIBUTIONS

YS and PJB wrote the manuscript.

ABBREVIATIONS

3α-diol, 3α-androstanediol

3α-HSD, 3α-hydroxysteroid dehydrogenase

3β-diol, 3β-androstanediol

3β-HSD, 3β-hydroxysteroid dehydrogenase

11β-HSD2, 11β-hydroxysteroid dehydrogenase 2

ACTH, adrenocorticotropic hormone

AR, androgen receptor

AVP, arginine vasopressin

BNST, bed nucleus of the stria terminalis

CBG, corticosteroid-binding globulin

CRH, corticotropin-releasing hormone

CSF, cerebrospinal fluid

DHDOC, dihydrodeoxycorticosterone

DHP, dihydroprogesterone

DHT, dihydrotestosterone

DOC, deoxycorticosterone

ER, estrogen receptor

GABA, γ-aminobutyric acid

GR, glucocorticoid receptor

HPA, hypothalamo-pituitary-adrenal

IGFBP-1, insulin-like growth factor binding protein-1

mPOA, medial preoptic area

mpPVN, medial parvocellular paraventricular nucleus

MR, mineralocorticoid receptor

NTS, nucleus of the solitary tract

p450scc, p450 side-chain cleavage enzyme

PPARα, peroxisome proliferator-activated receptor-α

PND, post-natal day

PR, progesterone receptor

PTSD, post-traumatic stress disorder

PVN, paraventricular nucleus

SHRP, stress hyporesponsive period'

StAR, steroidogenic acute regulatory protein

THDOC, tetrahydrodeoxycorticosterone

TSST, Trier Social Stress Test

VLM, ventrolateral medulla

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FIGURE LEGENDS

Figure 1: Sex differences in HPA axis responses to acute stress

Summary of the key factors underpinning greater activation of the HPA axis in female rodents (♀) than in males (♂) following acute stress. Abbreviations: ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; CORT, corticosterone; CRH, corticotropin-releasing hormone; GR, glucocorticoid receptor; mpPVN, medial parvocellular paraventricular nucleus; MR, mineralocorticoid receptor; + stimulatory action; -, inhibitory action.

Figure 2: The organisational-activational hypothesis of steroid action

The organisational-activational theory explains the role steroids play in determining sex differences in physiological stress responses. The organisational effects of steroids are exerted during critical periods of development such as during the fetal/postnatal testosterone surge or during puberty, where they act as morphogens to organise the neural circuitry. During adulthood, steroids act as allostatic mediators of stress, modulating gene expression or neuronal signalling, and augmenting or compensating for differences in neural circuitries that were determined earlier by the organisational effects of steroids. Sex differences in stress responses in adulthood are thus a result of an amalgation of the effects of steroids both in early life ("organisational" effects) and during adulthood ("activational" effects).

Figure 3: Biosynthetic pathways involved in steroidogenesis

Cholesterol enters the mitochondria (facilitated by the steroidogenic acute regulatory protein; StAR) and is cleaved by the p450 side-chain cleavage enzyme (p450scc) into pregnenolone. Pregnenolone can be converted to progesterone by 3β-hydroxysteroid hydrogenase (3β-HSD), which can subsequently be converted to deoxycorticosterone (DOC), then corticosterone. Corticosterone can be converted to the mineralocorticoid aldosterone by aldosterone synthase. Progesterone and DOC can also undergo two sequential A-ring reduction steps to form the 5α,3α-reduced steroids, allopregnanolone and tetrahydrodeoxycorticosterone (THDOC), respectively. These reduction steps are catalysed by the enzymes 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD), respectively. Reactions catalysed by 3α-HSD are reversible, hence allopregnanolone and THDOC can be

oxidised to DHP and DHDOC, respectively. Pregnenolone and progesterone can be converted to androgens by a two-step pathway, catalysed by the 17-hydroxylase and 17,20-lyase activities of a single enzyme, cytochrome P450c17. Testosterone can similarly be reduced to form dihydrotestosterone (DHT), and subsequently 3α-androstanediol (3α-diol) and 3β-androstanediol (3β-diol). Lastly, testosterone can also be converted to 17β-estradiol via the actions of aromatase (Cyp19a1). Chemical modifications such as sulfation may also change the properties and functions of steroids such as dehydroepiandrosterone (DHEA) and pregnenolone. Chemical structures of cholesterol, pregnenolone, progesterone, 5α-DHP and allopregnanolone are given as examples of the conversion by steroidogenic enzymes, where stereochemistry is an important aspect of each reaction.

Figure 4: Genomic and non-genomic action of steroids

Non-genomic actions of steroids in the brain are mediated through membrane-bound (m) steroid receptors or neurotransmitter receptors such as NMDA or $GABA_A$ receptors, resulting in rapid cellular changes involving the activation of intracellular signalling pathways (in the case of membrane-bound steroid receptors) or the changes in the membrane potential (in the case of neurotransmitter receptors). For genomic actions, steroids exert their effects via classical steroid receptor actions, where upon ligand binding, chaperone proteins dissociate, allowing two steroid-receptor complexes to dimerise. Dimerised receptors then enter the nucleus and bind to steroid responsive elements on genes to alter gene transcription and subsequently protein expression. Examples of steroid ligands that may bind to each receptor are listed below the receptors. Notably, steroids such as THDOC and allopregnanolone are allosteric modulators, therefore bind to a site away from the site of neurotransmitter binding. Abbreviations: 3β-diol, 3β-androstanediol; ALLO, Allopregnanolone; AR, androgen receptor; CORT, cortisol (in humans) or corticosterone (in rats and mice); DHEA, dehydroepiandrosterone; DHP, dihydroprogesterone; DHT, dihydrotestosterone; E2, estradiol; ER, estrogen receptor; GR, glucocorticoid receptor; (m), membrane bound; MR, mineralocorticoid receptor; PR, progesterone receptor; PregS, pregnenolone sulfate; Prog, progesterone; T, testosterone; THDOC, tetrahydrodeoxycorticosterone.

Figure 5: Steroid hormone profiles in the plasma and brain during pregnancy in the rat

a) Circulating concentrations of allopregnanolone, estradiol, progesterone and tetrahydrodeoxycorticosterone (THDOC); and b) concentrations of allopregnanolone, progesterone and THDOC in the cerebral cortex of pregnant rats. In each case hormone concentrations are expressed as a percentage of the maximum levels found in pregnancy. Data are derived from previously published studies (Concas *et al.*, 1998; Mann & Bridges, 2001). N.b. Estradiol concentrations in the brain across pregnancy are not available, possibly due to poor detection limits of the available assays (Toran-Allerand *et al.*, 2005; Meffre *et al.*, 2007).